SNP Resources: Finding SNPs Discovery and Databases

Mark J. Rieder, PhD

NIEHS Variation Workshop

January 30-31, 2006

SNP Resources: SNP discovery and cataloging

1. SNP discovery/genotyping: Genome-wide approaches
   - ✓ SNP Consortium
   - ✓ HapMap
2. The current state of SNP resources
3. Comprehensive SNP discovery
   NIEHS SNPs - Environmental Genome Project

SNP Databases - **How to Manual for finding SNPs**
In class - Tutorial

Genetic Markers: Overview

1. RFLPs (SNPs circa 1980)
2. Microsatellites (SSLP; di-, tri-, tetranucleotide repeats)
   - 1/50,000 bp
   - Linkage Studies 300-400 markers (~1 Mbp)
   - Multi-allelic/High heterozygosity/informative
   - Complex genotyping assays
3. Single Nucleotide Polymorphisms (SNPs)
   - Most frequent genetic variant (base substitutions)
   - 1/1000 bp (comparing randomly selected chromosomes)
   - Biallelic/less informative
   - Simplified genotyping platforms (+/- calling)

Development of a genome-wide SNP map: How many SNPs?

Table 1 • Occurrence of SNPs in the human population

Minimal allele frequency	Expected SNP number (millions)	Expected SNP frequency (bp)
1%	11.0	290
5%	7.1	450
10%	5.3	600
20%	3.3	960
30%	2.0	1,570
40%	0.97	3,280

Nickerson and Krugiyak, Nature Genetics, 2001

~ 10 million common SNPs (> 1- 5% MAF) - 1/300 bp

How has SNP discovery progressed toward this goal

Finding SNPs: Marker Discovery and Methods

SNP discovery has proceeded in two distinct phases:

1 - SNP Identification
   Define the alleles
   Map this to a unique place in the genom

2 - SNP Characterization
Determination of the genotype in many individuals
   Population frequency of SNPs

Finding SNPs: Marker Discovery and Methods

SNP Discovery has proceeded in two distinct

phases: 1 - SNP Discovery**/Characterization

2 - SNP Discovery/Characterization**

Finding SNPs: Marker Discovery and Methods

APBiotech - AstraZeneca - Aventis - Bayer - Bristol-Myers Squib - F.Hoffman-La Roche - Glaxo Wellcome

THE SNP CONSORTIUM LTD

IBM - Motorola - Novartis - Pfizer - Searle - SmithKline Beecham - Wellcome Trust

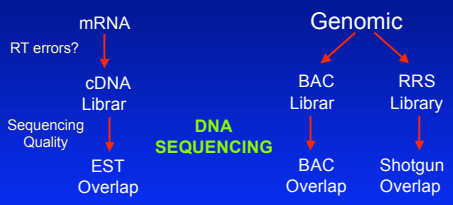
$ 45 Million 2 years (1999, 2001 2003)

Goals: Identify 300,000 SNPs and map 150,000 (April 1999)

Determine allele frequency of SNPs

If you dont have a reference genome how do you find SNPs?

Finding SNPs: Sequence-based SNP Mining



Sequence Overlap - SNP Discovery

GTTACGCCAATACAGATCCAGGAGATTACC
GTTACGCCAATACAGATCCAGGAGATTACC

Finding SNPs: Sequence-based SNP Mining

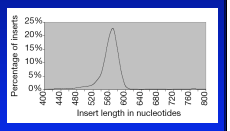
RRS = Reduced Representation Sequencin

Genomic DNA (multiple individuals)

RE to generate fragments

Clone DNA fragments into plasmid vector

Sequence and align and cluster



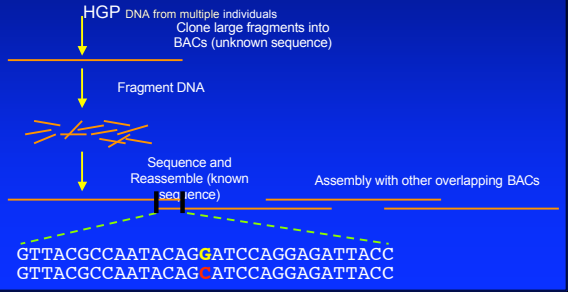
Altshuler, et al. Nature (2000)

GTTACGCCAATACAGGATCCAGGAGATTACC
GTTACGCCAATACAGCATCCAGGAGATTACC

From overlap SNPs

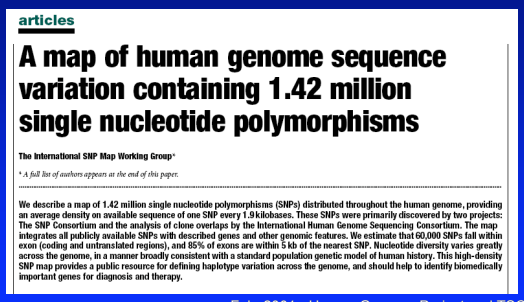
Finding SNPs: Sequence-based SNP Mining

BAC = Bacterial Artificial Chromosome
Primary vector for DNA cloning in the HGP



GTTACGCCAATACAGGATCCAGGAGATTACC
GTTACGCCAATACAGCATCCAGGAGATTACC

TSC and HGP: High Resolution SNP Map



articles

A map of human genome sequence variation containing 1.42 million single nucleotide polymorphisms

The International SNP Map Working Group*

*A full list of authors appears at the end of this paper.

We describe a map of 1.42 million single nucleotide polymorphisms (SNPs) distributed throughout the human genome, providing an average density on available sequence of one SNP every 1.9 kilobases. These SNPs were primarily discovered by two projects: The SNP Consortium and the analysis of clone overlaps by the International Human Genome Sequencing Consortium. The map integrates all publicly available SNPs with described genes and other genomic features. We estimate that 60,000 SNPs fall within exon (coding and untranslated regions), and 85% of exons are within 5 kb of the nearest SNP. Nucleotide diversity varies greatly across the genome, in a manner broadly consistent with a standard population genetic model of human history. This high-density SNP map provides a public resource for defining haplotype variation across the genome, and should help to identify biomedically important genes for diagnosis and therapy.

Feb. 2001 - Human Genome Project and TSC

Development of a genome-wide SNP map: How many SNPs?

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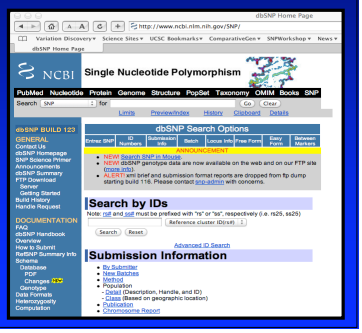
30%	2.0	1,570
40%	0.97	3,280

Nickerson and Kruglyak, Nature Genetics, 2001

~ 10 million common SNPs (> 1 - 5% MAF) - 1/300 bp Feb 2001- 1.42 million (1/1900 bp)

SNP Discovery: dbSNP database

dbSNP
-NCBI SNP database

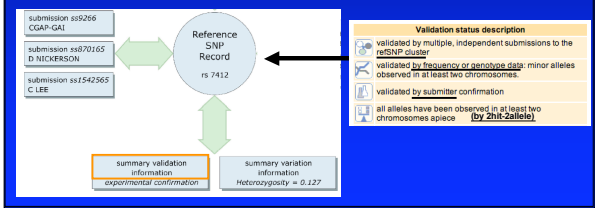


SNP data submitted to dbSNP: Clustering

dbSNP processing of SNP

SNPs submitted
By research communtiy
(submitted SNPs = ss#)

Unique mapping
to a genome location
(reference SNP = rs#)



Finding SNPs: Marker Discovery and Methods

SNP Discovery has proceeded in two distinct phases:

1 - SNP Identification**/Discovery

APBiotech - AstraZeneca - Aventis - Bayer - Bristol-Myers Squib - F.Hoffman-La Roche - Glaxo Wellcome
THE SNP CONSORTIUM LTD
IBM - Motorola - Novartis - Pfizer - Searle - SmithKline Beecham - Wellcome Trust

2 - SNP Discovery/Characterization**

International HapMap Project
Home | About the Project | Data | Publications

HapMap Project Proposed: Map more SNPs and genotype

International HapMap Project
Home | About the Project | Data | Publications

Participating Groups

Baylor College of Medicine (USA)
Beijing Genomics Institute (China)
Beijing Normal University (China)
Broad Institute of Harvard and MIT (USA)
Center for Statistical Genetics, University of Michigan (USA)
Chinese National Human Genome Center at Beijing (China)
Chinese National Human Genome Center at Shanghai (China)
Cold Spring Harbor Laboratory (USA)
Eubios Ethics Institute (Japan)
Health Sciences University of Hokkaido (Japan)
Hong Kong University of Science and Technology (China)
Howard University (USA)
Illumina (USA)
Johns Hopkins School of Medicine (USA)
McGill University & Génome Québec Innovation Centre (Canada)
ParAllele BioScience (USA)
Perlegen Science (USA)
RIKEN (Japan)
The Chinese University of Hong Kong (China)
The University of Hong Kong (China)
University of California, San Francisco (USA)
University of Ibadan (Nigeria)
University of Oxford (UK)
University of Oxford / Wellcome Trust Centre for Human Genetics (UK)
University of Tokyo (Japan)
University of Utah (USA)
Washington University, St. Louis (USA)
Wellcome Trust Sanger Institute (UK)

- **Increase SNP density over the first 6 - 12 months**
- **Ultimately produce a fine scale genetic map (HapMap) which would serve as a common resource for all biomedical reseseachers**
- **Genotype 600,000 SNPs genome-wide**
- **Four populations: CEPH (Europe), Yoruban (Africa), Japanese/ Chinese (Asian)**

HapMap SNP Discovery: Prior to Genotyping

Initiation of project planning (July 2001):
2.8 million SNPs (1.4 million validated) - 1/1900 bp
Nov 2003 - 5.7 million (2 million validated) - 1/1500 bp
Feb 2004 - 7.2 million (3.3 million validated) - 1/900 bp

Generate more SNPs:
Random Shotgun Sequencing

Genomic DNA
(multiple individuals)

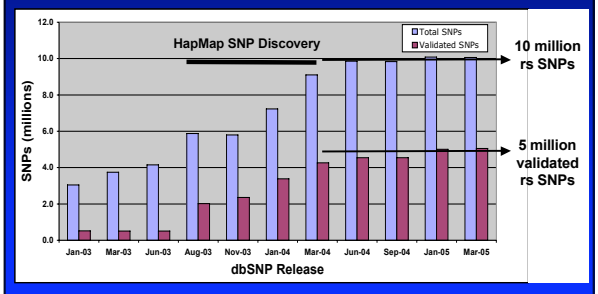
Sequence and align
(reference sequence)

TACGCCTATA TCAAGGAGAT
GTTACGCCAATACAGGATCCAGGAGATTACC

Draft Human Genome

Other Sources of SNPs:
Perlegen (Affymetrix chips) SNP data (chr22)
Sequence chromatograms from Celera project

HapMap Discovery Increased SNP Density and Validated SNPs



Development of a genome-wide SNP map: How many SNPs?

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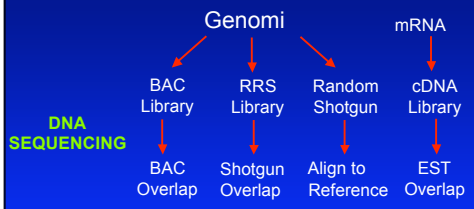
Nickerson and Kruglyak, Nature Genetics, 2001

~ 10 million common SNPs (> 1- 5% MAF) - 1/300 bp

Feb 2001 - 1.42 million (1/1900 bp)
Nov 2003 - 2.0 million (1/1500 bp)
Feb 2004 - 3.3 million (1/900 bp)
Mar 2005 - 5.0 million (validated - 1/600 bp)

When will we have them all?

Finding SNPs: Sequence-based SNP Mining

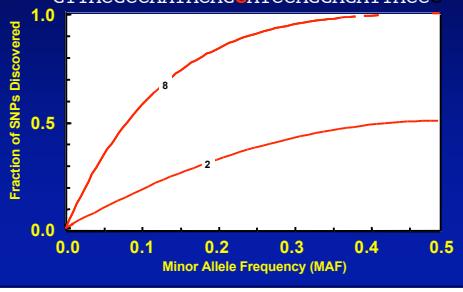


RANDOM Sequence Overlap - SNP Discovery

GTTACGCCAATACAG**G**ATCCAGGAGATTACC
GTTACGCCAATACAG**C**ATCCAGGAGATTACC

SNP discovery is dependent on your sample population size

2 chromosomes { GTTACGCCAATACAG**G**ATCCAGGAGATTACC
GTTACGCCAATACAG**C**ATCCAGGAGATTACC



SNP Characterization/Genotyping

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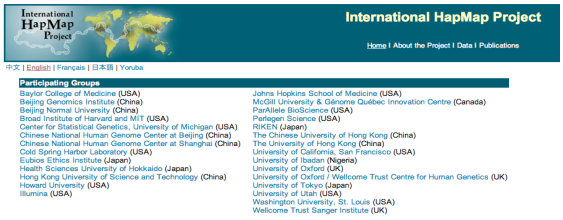
Nickerson and Kruglyak, Nature Genetics, 2001

~ 10 million common SNPs (>1- 5% MAF) - 1/300 bp

Mar 2005- 5.0 million (validated/mapped - 1/600 bp)

5.0/10.0 = 50% of all common SNPs (validated)!

HapMap Project Proposed: Map more SNPs and genotype

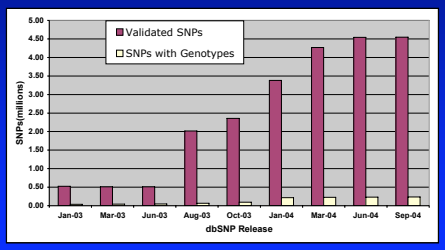


- **Genotype 600,000 SNPs genome-wide**
- **Four populations:**
  - **CEPH (CEU) (Europe - n = 90, trios)**
  - **Yoruban (YRI) (Africa - n = 90, trios)**
  - **Japanese (JPT) (Asian - n = 45)**
  - **Chinese (HCB) (Asian - n =45)**

Finding SNPs: Genotype Data Adds Value to SNPs HapMap Genotyping

- ✓ Confirms SNP as "real" and "informative"
- ✓ Minor Allele Frequency (MAF) - common or rare
- ✓ MAF in different populations
- ✓ Detection of SNP x SNP correlations (Linkage Disequilibrium)
- ✓ Determine haplotypes

Few SNPs in dbSNPs had Genotype Data



Perlegen Large-scale Genotyping Capacity

Whole-Genome Patterns of Common DNA Variation in Three Human Populations

David A. Hinds,[1] Laura L. Stuve,[1] Geoffrey B. Nilsen,[1] Eran Halperin,[2] Eleazar Eskin,[3] Dennis G. Ballinger,[1] Kelly A. Frazer,[1] David R. Cox[1]*

18 FEBRUARY 2005 VOL 307 SCIENCE

1.58 millions SNPs genotyped
71 individuals from 3 American populations
European, African and Asian ancestry

HapMap Completion

A haplotype map of the human genome

The International HapMap Consortium*

Inherited genetic variation has a critical but as yet largely uncharacterized role in human disease. Here we report a public database of common variation in the human genome: more than one million single nucleotide polymorphisms (SNPs) for which accurate and complete genotypes have been obtained in 269 DNA samples from four populations, including ten 500-kilobase regions in which essentially all information about common DNA variation has been extracted. These data document the generality of recombination hotspots, a block-like structure of linkage disequilibrium and low haplotype diversity, leading to substantial correlations of SNPs with many of their neighbours. We show how the HapMap resource can guide the design and analysis of genetic association studies, shed light on structural variation and recombination, and identify loci that may have been subject to natural selection during human evolution.

Nature - Oct 27 (2005)

- 2005-06-21: **HapMap public release #16c.1**
  This is the final Phase I data freeze as used in analyses for the upcoming primary HapMap publication (see **Data freezes** for more info). Also, note that with this release the abbreviation for the Han Chinese in Beijing population is changed to CHB. (See **Guidelines for Referring to HapMap Populations** for more info)
  Summary of genotyped SNPs:

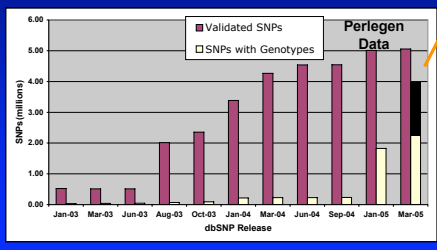
Populations	CEU	CHB	JPT	YRI
Genotyped SNPs	1,105,072	1,088,689	1,088,426	1,076,451

- 2005-10-24: **HapMap Public Release #19**
  Genotypes, frequencies and assays for phase I and phase II of the HapMap project are now available for **bulk download**. The files contain all phase I and II data combined.

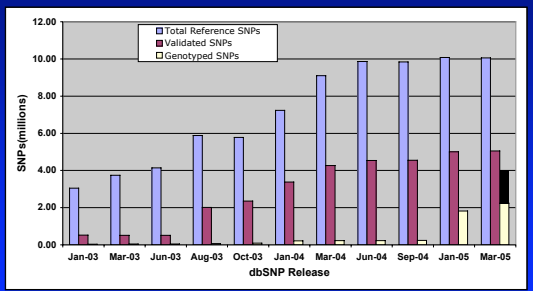
Populations	CEU	CHB	JPT	YRI
Total QC+ SNPs	3,901,408	3,903,524	3,902,623	3,806,920
Total Genotyped SNPs	5,894,684	5,812,990	5,812,990	5,857,466

HapMap + Perlegen

dbSNP: Increasing numbers of SNPs now have genotype data



Current State of dbSNP

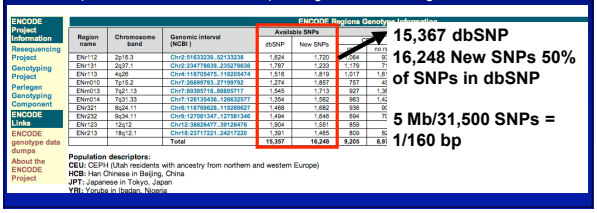


Many SNPs left to validate and characterize.

Increasing SNP Density: HapMap ENCODE Project

ENCODE ENCyclopedia Of DNA Elements
Catalog all functional elements in 1% of the genome (30 Mb)

10 Regions x 500 kb/region (Pilot Project)
David Altschuler (Broad), Richard Gibbs (Baylor)
16 CEU, 16 YRI, 8 HCB, 8 JPT
Comprehensive PCR based resequencing across these regions



15,367 dbSNP
16,248 New SNPs 50% of SNPs in dbSNP

5 Mb/31,500 SNPs = 1/160 bp

Development of a genome-wide SNP map: How many SNPs?

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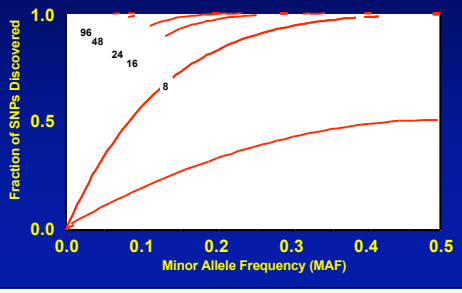
Nickerson and Kruglyak, Nature Genetics, 2001

~ 10 million common SNPs (>1- 5% MAF) - 1/300 bp **Mar 2005**- 5.0 million (validated - 1/600 bp)

~4.0 million validated SNPs with genotypes!
(HapMap confirmed, allele frequency/population, SNPxSNP correlations (LD), haplotypes)

SNP discovery is dependent on your sample population size

2 chromosomes { GTTACGCCAATACAG**G**ATCCAGGAGATTACC
GTTACGCCAATACAG**C**ATCCAGGAGATTACC



National Institute of Environmental Health Sciences
Environmental Genome Project
NIEHS SNPs
Search Site Go

Goal: Comprehensively identify all common sequence variation in candidate genes

Initial biological focus: Candidate environmental response genes involved in DNA repair, cell cycle, apoptosis, metabolism, cell signaling, and oxidative stress.

Approach: Direct resequencing of genes

Samples: PDR 90 ethnically diverse individuals representative of U.S. population (397 genes)
EGP95 95 samples from 4 ethnic groups (23 HapMap Asians, 22 HapMap Europeans, 15 HapMap Yorubans, 12 African Americans, 24 Hispanic) (170 genes)

Targeted SNP Discovery

Directed analysis: cSNPs

Complete analysis: cSNP and Haplotype Structure Analysis

•Generate SNP data from complete genomic resequencing
(i.e. 5' regulatory, exon, intron, 3 regulatory sequence)

Summary of NIEHS SNP genotypes in dbSNP

Table 1. Summary of genotype data contained in dbSNP

Data set	Genotypes	SNPs	Populations	Individuals	Average SNP density	Reference
HAPMAP	159,862,776	954,302	4	270	3149	(International HapMap Consortium 2003)
PERLEGEN	110,385,051	1,576,578	3	71	1938	(Hinds et al. 2005)
Affymetrix	6,189,466	125,778	6	116	24,029	(Kennedy et al. 2003)
TSC	4,932,382	19,048	17	1963	312,754	(International SNP Map Working Group 2001)
EGP	3,184,470	32,730	1	95	22,449	(Livingston et al. 2004)
PGA/UW	573,194	15,981	2	47	153,861	(Crawford et al. 2004)
IIPGA	176,162	3801	3	47	430,361	(Innate Immunity PGA, http://innateimmunity.net/)
NIHPDR	159,549	1982	1*	448	1,419,125	(Collins et al. 1998)
WICVAR	33,240	1462	1	130	2,011,277	
HG_BONN	24,522	320	1	143	5,284,550	(Freudenberg-Hua et al. 2003)

*The NIHPDR data contains a single mixed population.

Current numbers
554 genes sequenced
12.76 Mb scanned
75,580 genotyped SNPs identified
7 million genotypes deposited in dbSNP

Nov 2005 -Zaitlen et al. Genome Research 15:1594-1600

Development of a genome-wide SNP map: How many SNPs?

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Nickerson and Kruglyak, Nature Genetics, 2001

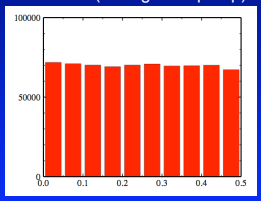
~ 10 million common SNPs (>1- 5% MAF) - 1/300 bp

NIEHS SNPs = 1/180 bp (n = 95, 4 pops)
HapMap ENCODE = 1/160 (n = 48, 3 pops)

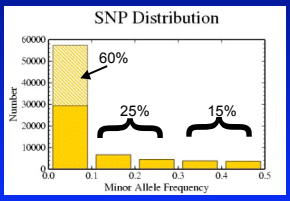
Comprehensive resequencing can identify the vast majority of SNPs in a region

SNP Discovery: dbSNP database

dbSNP (Perlegen/HapMap)



NIEHS SNPs



Minor Allele Freq. (MAF)

Rarer and population specific SNPs are found by resequencing

NIEHS SNPs Characterization

PDR = 90 ethnically diverse individuals representative of U.S. population (397 genes - ~55,000 SNPs)

Selection of informative (high frequency, coding, etc) SNPs to be genotyped in defined populations (~7600 SNPs)

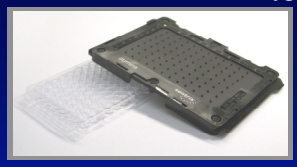
HapMap Populations

- European (CEU,n=60)
- African (YRI, n =60)
- Asian (HCB, n = 45 and JPT, n = 45)

Non-HapMap Populations

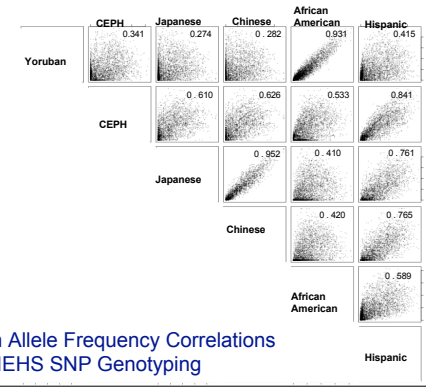
- Hispanic (n=60) African-American (n = 62)

Illumina NIEHS SNPs Genotyping



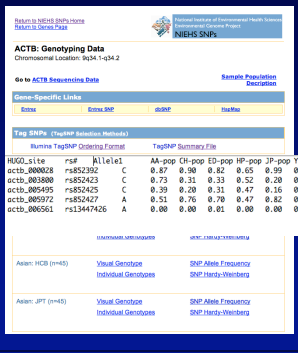
- Each well samples 1536 SNPs in one individual
- For each HapMap sample 5 x 1536 (7680 genotyped SNPs)
- 3,000,000 genotypes generated (total ~400 samples)

Array (1536)	Site Conversion Rate (%)	Average Site Coverage (%)	Concordance (%)
1	85	96.6	99.7
2	91	97.7	99.5
3	82	98.5	99.3



Population Allele Frequency Correlations
Illumina NIEHS SNP Genotyping

NIEHS SNPs Genotype Data



PDR (397 genes)

SNPs characterized in six different major populations.

Summary: The Current State of SNP Resources

- SNPs have been rapidly adopted as the genetic marker of choice.
- Approximately 10 million common SNPs exist in the human genome (1/300 bp).
- Random SNP discovery processes generate many SNPs (TSC and HapMap).
- Random approaches to SNPs discovery have reached limits of discovery and validation (1/600 bp; 50% SNP validation)
- Most validated SNPs (5 million) will be genotyped by the HapMap (3 pops)
- Resequencing approaches continue to catalog important variants (rarer)
- NIEHS SNPs has generated SNP data on >550 candidate genes and 75 K SNPs